

## SHORT COMMUNICATION

### Cold-hardiness in the wolf spider *Pardosa groenlandica* (Thorell) with respect to thermal limits and dehydration

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**Abstract.** The super-cooling point (SCP) of *Pardosa groenlandica* was found to be  $-10.54^{\circ}\text{C}$ , which is lower than for species from warmer climates, in agreement with predictions. There was no significant difference found between sexes. There were also no significant differences between the seasons, although there was a slight trend towards lower SCP temperatures in the winter months. Dehydration lowered SCP in *P. groenlandica*, with a mean depression of over  $3^{\circ}\text{C}$  in desiccated individuals. Specimens of *P. groenlandica* were able to move at temperatures as low as  $-2.3^{\circ}\text{C}$ . These data allow for comparisons with samples from other climatic regions, and reinforce predictions based on previous studies with a variety of arthropods, regarding the effects of hunger and desiccation on the SCP.

**Keywords:** Arachnida, cold tolerance, Lycosidae, sub-arctic, super-cooling

Arthropods that live in sub-zero temperatures for at least part of the year survive by one of two physiological and biochemical responses. At least for insects (Bale 2002), one way is tolerance of ice crystal formation in their bodies (freeze tolerance), and the other is avoidance of ice crystal formation (freeze avoidance). Ice crystal formation is avoided by super-cooling, which depresses the freezing point (Sinclair 1997; Renault et al. 2002). The super-cooling temperature depression is often used to determine cold-hardiness in invertebrates, and the temperature at which ice crystals form is called the super-cooling point SCP (Renault et al. 2002). Several physiological processes are known to cause super-cooling and SCP depression. These include accumulation of poly-ol compounds in the hemolymph (thus increasing the osmotic pressure), dehydration (also increasing osmotic pressure), synthesis of thermal-hysteresis protein, or evacuation or masking of ice-nucleation factors in the gut (Duman 1979; Somme 1982; Zachariassen 1982; Aunaas et al. 1983; Cannon & Block 1988; Danks 2005). Cold hardiness is particularly important in spiders as they are not known to be freeze-tolerant (Kirchner 1987). Among spiders there are a variety of strategies for surviving sub-zero temperatures (Schaefer 1977; Kirchner 1973, 1987). There are two main strategies to survive freezing temperatures by super-cooling temperature depression according to Kirchner (1987). One physiological strategy is to maintain a relatively constant SCP throughout the year. This strategy is found in species that have low ( $\text{SCP} = -3^{\circ} - -7^{\circ}\text{C}$ ) or medium ( $\text{SCP} = -7^{\circ} - -15^{\circ}\text{C}$ ) cold hardiness, by Kirchner's categories (Kirchner 1987). According to this idea, only species with high cold hardiness ( $\text{SCP} < -17^{\circ}\text{C}$ ) decrease their SCP as seasons proceed. The second physiological strategy is a lowering of the SCP as the temperature decreases through seasons. This strategy is found in species that have high cold hardiness.

In this study we used a common wolf spider (Lycosidae) *Pardosa groenlandica* (Thorell 1872) to test four research goals regarding the nature of cold hardiness in spiders. The species is found across northern Canada, Alaska, and Siberia, in terrain that ranges from exposed mountain slopes, to open plains, to stony coastlines (Dondale 1999). All the habitats share one feature: all experience several months at sub-zero temperatures, so that the species must be adapted for cold hardiness to remain active below  $0^{\circ}\text{C}$ . However, it is known that species of *Pardosa* from different regions, with differences in mean monthly temperatures, vary in their SCP. For example, *Pardosa*

species from Newcastle-upon-Tyne (UK) had higher SCP than a variety of Lycosidae from cooler climates (Kirchner 1987; Bayram & Luff 1993). Therefore, our first goal was to test whether *P. groenlandica* has a lower SCP than reported for the *Pardosa* species from warmer climates. We also determined the thermal limit of locomotion, at which temperature individuals are still active. The habitat of *P. groenlandica* would classify it as a species of medium cold hardiness (Kirchner 1987). Our second goal was to test whether *P. groenlandica* maintains the same SCP through seasons, as would be predicted by Kirchner (1987). Controlled dehydration is known to be one physiological response to decreasing the SCP in insects (Danks 2005) and in wolf spiders (DeVito & Formanowicz 2003). Since an increase in poly-ol concentration was proposed to be physiologically demanding in winter-active species (Duman 1979), dehydration would be a plausible alternative to increasing the osmotic pressure. Therefore, our third goal was to test whether dehydration contributes to cold hardiness and SCP depression in *P. groenlandica*. Finally, a difference in the SCP between sexes is known in some arthropods but is rare in spiders (Kirchner 1987; Salin et al. 2000). Our fourth goal was to test whether the SCP was different between male and female individuals of *P. groenlandica*.

## METHODS

**Study site.**—Spiders were collected from Fisherman's Cove in Eastern Passage, Nova Scotia ( $44^{\circ}36'\text{N}$ ,  $63^{\circ}29'\text{W}$ , elev. 5.18–6.09 m above sea level), with a mean annual temperature of  $6^{\circ}\text{C}$ . Seasonal temperature data were obtained from the nearby weather station at Shearwater, Eastern Passage, Nova Scotia. Mean over-wintering temperatures are December  $-1^{\circ}\text{C}$ , January  $-4^{\circ}\text{C}$ , February  $-4^{\circ}\text{C}$ , March  $0^{\circ}\text{C}$ , and April  $4^{\circ}\text{C}$ . The terrain was a rocky shore, with most rocks ranging from 10–30 cm in diameter. The sediment beneath the rocks was sandy and wet and acted as a barrier to the spiders. Spiders were located in and among the rocks from the water line up to and on the boardwalk. The rocks are covered in snow and ice through the over wintering period until April.

**Collecting spiders.**—Adult spiders were collected as needed between June and December 2005 with no specific time interval between samplings. This period covers the warmest months and extends through the cooling months to winter. Individual spiders were captured and placed into 14 ml glass vials. In favorable weather (see below) about 20 spiders could be collected in 30 min by 3 or 4 people. No biases were made towards size or sex. Collected spiders

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Table 1.—Seasonal variation in super-cooling points (SCP) of *Pardosa groenlandica*.

Time period	Mean field temperature range (° C)	SCP (° C) (mean $\pm$ SD)	n	SCP range (° C)		
				Max.	Min.	P
June–July	9–22	$-9.8 \pm 2.29$	20	–6.6	–12.8	0.164
August–September	10–22	$-10.8 \pm 1.77$	21	–7.5	–15.0	0.618
October–December	–5–13	$-11.7 \pm 2.9$	9	–7.5	–15.3	0.265
Overall		$-10.54 \pm 2.43$	50	–6.6	–15.3	

were maintained at 22° C without food, and experiments were performed within two hours of collection. Voucher specimens have been deposited in the Natural History Museum, Department of Biology, Dalhousie University.

**Determination of super-cooling point.**—Spiders were placed in a 2 ml tube and held in place using a 4 cm<sup>2</sup> piece of paper towel. A thermocouple (Virtual Instruments Atlantic Low Temperature Systems, Austin, Texas) was fed through a small hole in the lid of the tube and was attached to the spider via a minute amount of petroleum jelly. The thermocouple led to a digital reader which stored temperature data. Spiders were frozen one at a time in an Endocal Refrigerated Cooling Bath (NESLAB, Newington, New Hampshire) set at  $20 \pm 0.05$ ° C. The bath temperature was then decreased at a rate of 1° C per minute while an electronic graph was made of the temperature. The SCP was defined by a sharp increase in temperature recorded. Readings were ceased once the temperature recorded returned to a point lower than the SCP. After each trial, the temperature of the cooling bath was returned to 20° C for the next trial. SCP means were compared across seasons. SCP were analyzed using ANOVA (Zar 1984) and significance tested at 95% confidence. Throughout this paper, means are reported  $\pm$  SD.

**Effect of dehydration on super-cooling point.**—In September 2005, 29 spiders were randomly selected regardless of life stage, size, or sex and placed into one of two groups: The 14 spiders in the control group were individually contained in 22.1 cm<sup>3</sup> vials with the lid on, but the lid was temporarily removed once per day to allow air renewal. The 15 spiders in the treatment group were also contained in the same size vial, but instead of a lid each vial was covered with cheesecloth held in place with an elastic band. All spiders were individually weighed using an electronic balance accurate to 0.001 g and again after 3 days. The SCP experiment was then performed as described above. Comparison of weight lost and SCP between the control and experimental groups were carried out using ANOVA (Zar 1984) and significance tested at 95% confidence.

**Thermal limit of locomotion.**—In October 2005, 20 spiders were individually placed in 14 ml vials. A hole was made in the top of the vial with a scalpel through which a thermocouple was fed. The vials were placed in a programmable temperature-controlled cooling bath which cooled at a rate of  $1 \pm 0.2$ ° C per 3 min. The starting temperature was set at 0° C. At each  $-1$ ° C interval, measured by a thermocouple thermometer (Hanna Instruments Model HI 93530, Laval, Québec, Canada) the spiders were observed and qualitative notes were taken on the spider's ability to move. The experiments were ended at the temperature the spider was no longer observed to move, and this temperature was recorded as the thermal limit of locomotion for that individual.

## RESULTS

**General observations.**—Spiders were about 1 cm in body length not including the legs. Spiders were present in abundance regardless of temperature during the collection period with the exception of rainy and/or windy days. Specimens were collected mostly between and under rocks, with more being found on the boardwalk on colder days. Spiders were only found in high numbers on sunny days, with fewer being found as cloud cover and/or wind intensity increased.

**Determination of super-cooling point (SCP).**—A total of 50 spiders was tested between June 6<sup>th</sup> and December 5<sup>th</sup> 2005. The average SCP was  $-10.5 \pm 2.4$ ° C, range =  $-15.3$  –  $-6.6$ ° C (Table 1). None of the individuals survived, indicating that this species was not tolerant of ice crystal formation below the SCP. There was no significant difference in the mean between seasons ( $P > 0.05$ ). A random sample of 12 female and 12 male spiders collected in September 2005 were sexed for comparison of their SCP. The SCPs of males and females were  $-11.4 \pm 2.7$ ° C and  $-10.5 \pm 1.3$ ° C, respectively. Analysis with ANOVA showed no significant difference between SCP by sex ( $P = 0.322$ ).

**Effect of dehydration on SCP.**—Twenty eight spiders were randomly assigned to either the control group or the dehydration group. As shown in Table 2, the dehydration group lost more mass than did the control group ( $-0.015 \pm 0.011$  g versus  $-0.002 \pm 0.003$  g). Analysis using a t-test showed that the mean SCP of the control group was not different from the mean SCP of the species ( $-10.5$ ° C) ( $P = 0.957$ ). The dehydration treatment resulted in a SCP 3.3° C lower than the control group. Analysis with ANOVA shows this to be a significant difference ( $P = 0.001$ ).

**Thermal limit of locomotion.**—The mean temperature at which the randomly chosen spiders ( $n = 20$ ) could no longer move was  $-2.4$ °  $\pm$   $0.9$ ° C, range =  $-1.0$ ° C –  $-4.0$ ° C. All spiders slowed down at 0° C and regained mobility after the experiment when 1 min after their body temperatures returned to normal. There did not appear to be any immediate detrimental effect on their health.

## DISCUSSION

**Super-cooling point and thermal limit of locomotion.**—The wolf spiders *P. pullata* (Clerck 1757) and *P. amentata* (Clerck 1757) from Newcastle-upon-Tyne (UK) were shown to have a higher super-cooling point (SCP) (Bayram & Luff 1993) than spiders that lived in cooler climates (Kirchner 1973; Schaefer 1977). Our results show that *P. groenlandica* from a cooler climate had lower SCP. Overall these results support the idea that SCP is lower in spider species from colder habitats as proposed by Kirchner (1987). At least, our results confirm this trend within the genus *Pardosa*. With a mean SCP of  $-10.54$ ° C, *P. groenlandica* falls into the medium range of cold-hardiness predicted by its habitat (Kirchner 1987). This finding is comparable to other species of lycosids, for example, *Arctosa perita* (Latreille 1799) and *Pirata piraticus* (Clerck 1757) with SCP of  $-11.0$ ° C and  $-14.5$ ° C, respectively.

The mean thermal limit of locomotion found in *Pardosa groenlandica* was  $-2.4$ ° C. This is not an unusual value, as there are many species of spiders capable of locomotion below 0° C. At temperatures below 0° C it is less likely to find spiders in the open because they move more slowly and they would become more vulnerable to predation. It is likely that the SCP is lower than the thermal limit of locomotion because spiders can still become active during brief warm spells, especially in more sheltered spaces among the rocks. While the protocol used to determine the thermal limit of locomotion is relatively standard, it places the spiders in an artificial setting where they do not have much space to move. Wolf spiders are known to exhibit thermoregulatory behavior in addition to their resistance to cold by super-cooling (DeVito & Formanowicz 2003). It



Table 2.—Effect of dehydration on super-cooling point (SCP) in *Pardosa groenlandica*.

Treatment	Start weight (g, mean $\pm$ SD)	End weight (g, mean $\pm$ SD)	Weight loss (g, mean $\pm$ SD)	SCP (° C, mean $\pm$ SD)	n	P
Control	0.152 $\pm$ 0.055	0.151 $\pm$ 0.057	0.001 $\pm$ 0.007	10.6 $\pm$ 1.9	14	0.001
Dehydrated	0.142 $\pm$ 0.175	0.129 $\pm$ 0.178	0.013 $\pm$ 0.0116	13.9 $\pm$ 2.5	14	

may be that, under these conditions, heat that would have been generated by moving was not possible.

**SCP change with seasons.**—As *P. groenlandica* is a species with medium cold-hardiness, we predicted that the SCP of this species would not differ significantly from season to season (Kirchner 1987). The results (Table 1) support the hypothesis, although a trend was observed in the data. During the October to December period of sampling there was a noticeable, but statistically insignificant, tendency towards lower SCP in the spiders, with points reaching as low as  $-15.3^{\circ}\text{C}$  and with only three findings below  $-12.0^{\circ}\text{C}$ . The results indicate that *P. groenlandica* demonstrates aspects of both of Kirchner's (1987) cold-hardiness strategies. However, there are probably intermediate species that do not fit into these categories. For example *Pirata piraticus*, with a SCP of  $-14.5^{\circ}\text{C}$ , does not meet the criterion of the high-cold-resistance category (i.e.,  $\text{SCP} < -17^{\circ}\text{C}$ ), but shows a depressed SCP during the winter compared to summer months (Kirchner 1987).

**Effect of dehydration on super-cooling point.**—Wolf spiders are known to control evaporative water loss and are capable of water uptake directly from the soil (DeVito & Formanowicz 2003). This is not unusual in soil-dwelling organisms (Adl 2003), and it was recently observed in the oribatid mite *Phauloppia* sp. (Sjursen & Sømme 2000). Moisture from the sand below the stones on this coastline is probably an important source of water for *P. groenlandica*. The results support our hypothesis that dehydration decreased the SCP and therefore increased cold hardiness in this species by  $-3.3^{\circ}\text{C}$  (Table 2). Since the spiders in the dehydration experiment were not fed for three days, it is possible, but unlikely, that hunger altered the SCP of either the control or the dehydration groups. However, the mean SCP of the control group in this experiment ( $-10.6^{\circ}\text{C}$ ) was not significantly different than the mean SCP determined in the previous experiment ( $-10.5^{\circ}\text{C}$ ) by two tailed t-test with  $P = 0.0296$  at 95% confidence (Tables 1, 2).

**SCP between males and females.**—Wolf spiders do not usually show a difference in SCP between sexes (Kirchner 1987). Results from a related wolf spider *Pardosa pullata* also determined that the SCP did not differ between the sexes (Bayram & Luff 1993). However, it is not known how generalized this statement is for wolf spiders, especially when there are morphological differences between sexes, as in *P. groenlandica*. Other arthropod species with marked morphological differences between sexes sometimes show differences in SCP, as in the lesser mealworm (Salin et al. 2000). The results obtained from this study support the hypothesis that there is no significant difference among the sexes in *P. groenlandica* with regards to SCP, and this is consistent with data available to date on wolf spiders.

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#### LITERATURE CITED

Adl, M.S. 2003. Ecology of Soil Decomposition. CABI, Wallingford, UK. 364 pp.

- Aunaas, T., J.G. Baust & K.E. Zachariassen. 1983. Ecological studies on arthropods from Spitsbergen. Polar Research 1:235–240.
- Bale, J.S. 2002. Insects and low temperatures: from molecular biology to distributions and abundance. Philosophical Transactions of the Royal Society (London) 357:849–862.
- Bayram, A. & M.L. Luff. 1993. Cold-hardiness of wolf-spiders (Lycosidae, Araneae) with particular reference to *Pardosa pullata* (Clerck). Journal of Thermal Biology 18:263–268.
- Cannon, R.J.C. & W. Block. 1988. Cold tolerance of microarthropods. Biological Reviews 63:23–77.
- Danks, H.V. 2005. Key themes in the study of seasonal adaptations in insects. I. Patterns of cold hardiness. Applied Entomology and Zoology 40:199–211.
- DeVito, J. & D.R. Formanowicz. 2003. The effects of size, sex, and reproductive condition on thermal and desiccation stress in a riparian spider (*Pirata sedentarius*, Araneae, Lycosidae). Journal of Arachnology 31:278–284.
- Dondale, C.D. 1999. Revision of the *groenlandica* subgroup of the genus *Pardosa* (Araneae: Lycosidae). Journal of Arachnology 27:435–448.
- Duman, J.G. 1979. Sub-zero temperature tolerance in spiders: the role of thermal hysteresis factors. Journal of Comparative Physiology 131:347–352.
- Kirchner, W. 1973. Ecological aspects of cold tolerance in spiders (a comparative study). Pp. 271–279. In Effects of Temperature on Ectothermic Organisms: Ecological Implications and Mechanisms of Compensation. (W. Wieser, ed.). Springer-Verlag, Berlin.
- Kirchner, W. 1987. Behavioural and physiological adaptations to cold. Pp. 66–77. In Ecophysiology of Spiders. (W. Nentwig, ed.). Springer-Verlag, Berlin.
- Renault, D., C. Salin, G. Vannier & P. Vernon. 2002. Survival at low temperatures in insects: what is the ecological significance of the super-cooling point? CryoLetters 23:217–228.
- Salin, C., D. Renault, G. Vannier & P. Vernon. 2000. A sexually dimorphic response in super-cooling temperature, enhanced by starvation, in the lesser mealworm *Alphitobius diaperinus* (Coleoptera: Tenebrionidae). Journal of Thermal Biology 25:411–418.
- Schaefer, M. 1977. Winter ecology of spiders (Araneida). Zeitschrift für Angewandte Entomologie 8:113–134.
- Sinclair, B.J. 1997. Seasonal variation in freezing tolerance of the New Zealand alpine cockroach *Celatoblatta quinque-maculata*. Ecological Entomology 22:462–467.
- Sjursen, H. & L. Sømme. 2000. Seasonal changes in tolerance to cold and desiccation in *Phauloppia* sp. (Acari, Oribatida) from Finse, Norway. Journal of Insect Physiology 46:1387–1396.
- Sømme, L. 1982. Supercooling and winter survival in terrestrial arthropods. Comparative Biochemistry and Physiology 73A: 519–543.
- Zachariassen, K.E. 1982. Nucleating agents in cold hardy insects. Comparative Biochemistry and Physiology 73A:557–562.
- Zar, J.H. 1984. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, New Jersey. 718 pp.

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